REMARKS

Claims 1-5, 7-12, and 14-64 are currently pending in the application. Claims 1 and 7, 10, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59 and 62 are amended. The amended claims find support in the specification, and no new matter has been added.

Specifically, claim 7 has been amended only with respect to its punctuation, a comma being added in line 2.

Claims 10, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59 and 62 have been amended to correct a typographical error of the second amino acid in SEQ ID NO:167. The second amino acid "R" of SEQ ID NO:167 has been replaced with the amino acid "P". There is no new matter as this change in the sequence listing now accurately reflects the sequence correlated with the SEQ ID NO:167 tag in the sequence listing. Claims reciting SEQ ID NO:167 always depend directly from a claim which recites SEQ ID NO:4. SEQ ID NO:4 is a 26mer, the first 16 amino acids of which are identical to SEQ ID NO:167. The second amino acid in SEQ ID NO:4 is "P".

Claim 1 is drawn to a method of detecting a precancerous lesion resulting from a mucosal papilloma virus infection in an organism. Claim 1 has been amended to include an interpreting step in order to more clearly delineate its inventive steps.

Claims Rejections – 35 U.S.C. Section 112, second paragraph

The Office Action states that claims 1-5, 7-12, and 14-64 are rejected under 35 U.S.C. Section 112, second paragraph, as being incomplete for omitting essential steps, such as an omission amounting to a gap between the steps, and further states that the omitted step(s) are an interpreting step which relates binding of the E4-detecting molecule to the detection of a precancerous legion.

Solely for the purposes of more clearly defining the invention, Applicant has amended independent claim 1, from which the remaining claims depend, to include an interpreting step which relates binding of the E4-detecting molecule to the detection of a precancerous legion. Specifically, claim 1 as amended now includes the following interpreting step: "wherein specific binding by said molecule to mucosal papilloma virus-E4 protein indicates the presence of mucosal papilloma virus-E4 protein expression and the detection of vegetative mucosal

papilloma viral DNA replication, thereby detecting a precancerous lesion resulting from a mucosal papilloma virus infection in an organism".

Support for this newly added interpretive steps is found throughout the specification, including on page 13, lines 9-12, where it is disclosed "It has surprisingly been found that E4 expression correlates strongly with vegetative DNA replication in HPV-infected cells, making detection of E4 expression a particularly appropriate indicator of HPV infection, and thus particularly useful in screening for precancerous cervical lesions."

In view of newly amended claim 1, Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claims Rejections – 35 U.S.C. Section 102

The Office Action states that claims 1-5, 7-8, 12, 14-30 and 64 are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Palefsky et al.

Applicant respectfully traverses the rejection. In rejecting the instant claims, the office action directs attention to Table 1 and Figure 2A of the Palefsky et al. reference.

Applicant contends that Figure 2A of Palefsky et al. does not teach the instantly recited method of detecting a precancerous lesion by detecting vegetative mucosal papilloma viral DNA replication, as required by the newly added interpretive step of the instant claims:

> "wherein specific binding by said molecule to mucosal papilloma virus-E4 protein indicates the presence of mucosal papilloma virus-E4 protein expression and the detection of vegetative mucosal papilloma viral DNA replication, thereby detecting a precancerous lesion resulting from a mucosal papilloma virus infection in an organism. ".

Figure 2A of the Palefsky et al. indicates no such interpretive step but instead merely illustrates that an antibody generated using a peptide immunogen consisting of a 15 amino acid peptide from the E4 protein of HPV16 is able to distinguish between the E4 protein from HPV16 versus and an E4 protein from HPV 6. A figure that teaches antibody specificity and that says nothing about the recited method of detecting a precancerous lesion, does not anticipate the recited method of detecting a precancerous lesion.

Neither does Table 1 of the Palefsky et al. reference teach the instantly recited method of detecting a precancerous lesion by detecting vegetative mucosal papilloma viral DNA replication as indicated by E4 expression. Table 1 of the Paefsky et al. reference demonstrates the presence of the E4 protein in a fraction of normal tissue, as well as in various stages of cancer. Since the E4 antigen was detected in at least some of the samples from both normal tissues as well as cancerous tissues, this table does not support a method of detecting a precancerous legion by detecting E4 expression. Further, said Table 1 does not indicate anything about the presence of vegetative mucosal papilloma viral DNA replication.

Because independent claim 1 is not anticipated by the Palefsky et al. reference, its dependent claims are similarly not anticipated by the reference. Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claims Rejections – 35 U.S.C. Section 102

The Office Action states that claims 1-15, 7-12, and 14-64 are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Doorbar et al., and directs attention to Figures 2-4 of the Doorbar et al. reference.

Applicant respectfully traverses the rejection.

The Office Action refers to Figures 2, 3 and 4 of the Doorbar reference as evidence that it anticipates the claimed invention. Applicants submit that Figure 2 shows that monoclonal antibodies to HPV E4 bind to HPV 16 E4 protein expressed in bacteria, Figure 3 shows the results of epitope mapping of the monoclonal antibodies against a panel of synthetic HPV 16 peptides, and Figure 4 shows the localization of E4 protein in a lesion known to be HPV 16 positive. Neither these figures, nor the reference as a whole, teaches the recited method of detecting a precancerous lesion resulting from a mucosal papilloma virus infection in an organism by detecting vegetative viral DNA replication comprising the detection of the E4 protein. The Doorbar reference does not even suggest that the molecules (antibodies) taught that specifically bind to E4 are useful for detecting a precancerous lesion resulting from a mucosal papilloma virus infection in an organism. Rather, the reference actually teaches away from the use of their antibodies for detection, since only 1 of 16 CIN biopsies already known to be HPV

16 positive stained positive for E4 expression with those antibodies (page 355, column 1, second paragraph).

Further, Doorbar et al. teach do not teach a method of detecting a precancerous lesion by detecting vegetative mucosal papilloma viral DNA replication as indicated by E4 expression, as required by newly amended independent claim 1. Accordingly, the instant claims are not anticipated by Doorbar et al., and Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claims Rejections – 35 U.S.C. Section 102

The Office Action states that claims 1-5, 7, 8, 12, 14-30 and 64 are rejected under 35 U.S.C. 102(e) as being clearly anticipated by U.S. 5,415,995, ("the '995 patent") and directs attention to claim 1, parts (13) and (14).

Applicant respectfully traverses the rejection on the grounds that the '995 patent does not teach the entire invention as more clearly described in the instant newly amended independent claim 1.

Claim 1, parts (13) and (14) of the '995 patent recite the following:

> "A method for indicating the presence of human papilloma virus in a clinical sample which comprises the steps of:
>
> (a) providing an antibody-containing composition, said composition containing antibody immunospecific for an epitope having an amino acid sequence selected from the group consisting of . . .
>
> (13) Asp-Gln-Asp-Gln-Ser-Gln-Thr-Pro-Glu-Thr-Pro (SEQ ID NO:13), representing residues 48-58 of E4;
>
> (14) Gly-Ser-Thr-Trp-Pro-Thr-Thr-Pro-Pro-Arg-Pro-Ile-Pro-Lys-Pro (SEQ ID NO:14), representing amino acids 20-34 of E4;. . .
>
> (b) contacting said clinical sample with said composition under antibody-antigen complex formation conditions,
>
> (c) detecting the formation of antibody-antigen complex resulting from the contacting of step (b), and
>
> (d) relating the detection of complex of step (c) to the presence in the clinical sample of human papilloma virus.".

These interpretive step of the method claims for indicating the presence of human papilloma virus of the '995 patent highlighted by the examiner, are not identical to that of the

instantly claimed newly amended method claims, which are directed to a method of detecting a precancerous lesion resulting from a mucosal papilloma virus infection. As such, the '995 patent not teach the instantly recited method of detecting a precancerous lesion comprising contacting the cells with a molecule that binds specifically to mucosal papilloma virus- E4 protein, nor does the '995 patent disclose correlating E4 expression to vegetative mucosal papilloma viral DNA replication.

Further, the '995 patent provides no data using an antibody or any other molecule that binds specifically to mucosal papilloma virus- E4 protein. Even after the time of issuance of the '995 patent, the state of the art was unpredictable regarding which if any of the papillomavirus early proteins could be detected in SSC lesions or could be detected in CIN lesions at various stages. The instant specification addresses such unpredictability at page 3, lines 1 – 3 and lines 5 - 17, as follows:

> "Thus, for example in Fields Virology (Fields et al, [Eds.] Virology Vol. 2, p2099, 3rd Edn. (1996) Raven Press, New York), an authoritative virology text book, it is stated that "Diagnosis of an HPV type in a tissue requires nucleic acid hybridization studies",

and

> "In contrast, screening for cervical carcinoma by detection of expression of HPV polypeptides has generally been disregarded, being considered unsuitable for a number of reasons, primarily because of the difficulty in obtaining suitable reagents and, more significantly, many HPVs produce very little virus protein in mucosal infections, making detection difficult, uncertain and unreliable. Thus, in Fields Virology (cited above) it is stated that "immunologic detection of viral capsid antigens" is "of limited value". The possibility of immunologic detection of other viral antigens is not even considered. If one were to develop a screening method based on detection of expression of viral proteins, the most likely choice of target would be those proteins which are best-characterized, such as L1 or L2. The function of E4 protein is at present unknown. Its expression pattern in cervical lesions has not been determined conclusively in the prior art so the molecule has not been an obvious choice for selection as a target for detecting HPV infection."

This unpredictability is also evident in the data presented in the '995 patent, for example, the data regarding the binding of cellular lesions by antibodies directed to only protein E6. Table 1 of the '995 patent shows the results of staining CIN lesions of various stages and SSC lesion by antibodies directed to four distinct peptides of the same protein, E6. Table 1 shows that even antibodies directed to the same protein bound differentially to CIN lesions of various stages, and further that only three of these four peptides bound to SSC lesions. The '995 patent provides no

basis for these variable results nor guidance showing which, if any, lesions will bind antibodies directed to the E4 protein epitopes.

Further, the '995 patent provides no data showing binding of with antibodies or any other molecule to E4 on the surface of any cells, including precancerous lesions. In view of this lack of guidance in the disclosure of the '995 patent, and in view of the unpredictability of E4 protein expression in CIN and SSC lesions at the time of issuance of the '995 patent as evidenced by Fields Virology discussed above, Applicant submits that the '995 patent does not teach or suggest the instantly claimed method of detecting a precancerous lesion resulting from a mucosal papilloma virus infection in an organism comprising contacting the cells with a molecule that binds specifically to mucosal papilloma virus E4 protein.

Further the '995 patent does not teach a method of detecting a precancerous lesion resulting from a mucosal papilloma virus infection comprising the newly added limitation to claim 1 of detecting vegetative mucosal papilloma viral DNA replication. Because the '995 patent does not teach a method of detecting a precancerous lesion, comprising detecting vegetative mucosal papilloma viral DNA replication as indicated by E4 protein expression as required by the instant claims, the referenced '995 patent does not anticipate the invention recited by the instant claims.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claims Rejection 35 U.S.C. 102

The Office Action states that claims 1-5, 7-8, 12, 14-30 and 64 are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Crum et al. (1990), *Virology* 178:238-246.

Applicant respectfully traverses the rejection on the grounds that 1) the cited reference does not teach the claimed method which comprises a molecule that <u>specifically</u> bind to mucosal papilloma virus-E4 protein, and 2) the cited reference does not teach a method comprising the newly added limitation to claim 1 of <u>detecting vegetative mucosal papilloma viral DNA replication</u>.

The examiner characterizes Crum et al. as teaching the screening of 150 potentially infected samples with anti-E4 antiserum, and detecting cytoplasmic binding in samples with precancerous lesions. Like Doorbar, Crum et al. found E4 in only a fraction of the C1N1 lesions that they looked at. Crum et al. detected E4 in only 5 out of 150 cases, where Doorbar detected E4 in 1 out of 10 cases. Both Crum's and Doorbar's failure to detect E4 in so many cases, indicates that that E4 has little use as a diagnostic reagent for the detection of a precancerous lesion resulting from a mucosal papilloma virus infection in an organism, and thus teaches away from the instantly claimed method. Indeed, Applicant notes the disclaimer regarding the questionable specificity of the antibodies used for the screening in the last paragraph of the cited reference:

> "Determining whether the E4 antigenic determinants recognized in the tissue by the antisera used in this study are truly specific for HPV-16 E4 will require mapping of the epitope(s) identified by the sera and a comparison with sequences of other HPV types." page 244, column 2, last sentence.

That the specificity of the antibody is in doubt is further evidenced by the first sentence of the same paragraph which states:

> "It is important to stress that in the absence of a positive control for HPV-16 E4 expression, the above evidence is by necessity indirect, and based upon interpretation of immunohistochemical data with its inherent limitations." page 244, column 2.

Because the cited reference does not teach a method of detecting a precancerous lesion comprising the use of a molecule which specifically binds mucosal papilloma virus-E4 protein, the reference does not anticipate claim 1, or its dependent claims.

The examiner has not applied this reference as art to claims which recite SEQ ID NO:4, 167 or 168 on the belief that these sequences lie slightly outside of the E4 region shown by Figure 1 of Crum. There are fundamental differences in the antisera disclosed in the instant application with respect to the antisera taught by Crum et al. The antibody taught by Crum et al. was generated to an E4 protein fragment (bases 3401-3697 of the 16E4 sequence) fused to TrypE. The E4 sequence encoded by DNA fragment 3401-3697 is: TTPPRPIPKPSPWAPKKHRRLSSDQDQSQTPETPATPLSCCTETQWTVLQSSLHLTAHTK DGLTVIVTLHP, which shares sequence overlap with the instantly recited sequences.

· However, the instantly disclosed antibody which was directed to the full length E4 fused to GST. The full E4 sequence that was used to prepare the monoclonal antibodies is shown below:

MADPAAATKYPLLKLLGSTWPTTPPRPIPKPSPWAPKKHRRLSSDQDQSQTPETP ATPLSCCTETQWTVLQSSLHLTAHTKDGLTVIVTLHP.

The GST component generally forms a separate well-ordered domain. Structural analysis has suggested that the N terminus of the E4 protein is necessary for correct folding, and may explain why the instantly disclosed antibodies are much more effective in detecting E4 protein and why Crum et al. did not reach the conclusion that E4 could be useful as a diagnostic marker for detecting a precancerous lesion resulting from a mucosal papilloma virus infection . So even within the regions where both antibodies bind, there are large differences in affinity between the instantly disclosed antibodies and the referenced antibodies.

It is unlikely that anyone knowledgeable in the field would have considered developing E4 as a diagnostic marker in the face of Crum's comments on page 241 that 'it was not possible to distinguish striking differences between E4 and L1 antigen distribution', which suggests to the reader that E4 staining is not significantly different from L1 staining in its distribution' (reference to this is also made on page 244 column 1). Similar studies on HPV11 ((Brown, Bryan et al. 1995) had also suggested (mistakenly see (Peh, Middleton et al. 2002; Middleton 2003)) that in E4 and L1 co-localise. This indicates that E4 detection offered no advantage over L1 staining, which was already considered to be of limited use as a diagnostic for detecting a precancerous lesion resulting from a mucosal papilloma virus infection .

Since Crum et al. was unable to observe better results with E4 than L1 using his polyclonal antibodies, there is no specific teaching in Crum et al. to use E4. In contrast, the instant application and in the publication that followed (Doorbar 1997) clearly show that E4 patterns are distinct from L1 and that the patterns are different in lesions of different grade (i.e. E4 can be preserved even when expression of E4 is lost. This demonstrates that E4 has a utility as a marker that is not offered by L1.

However even if there is some overlap in the immunogen taught by Crum and instantly disclosed, the preamble and interpreting steps of the instantly claimed methods distinguish the instantly claimed invention from that of Crum et al. Therefore, even those claims which recite SEQ ID NO:4, 167 or 168 and were not cited in the instant rejection are not anticipated by Crum et al. Crum et al. does not teach a method of detecting a precancerous lesion resulting from a mucosal papilloma virus infection comprising the newly added limitation to claim 1 of detecting vegetative mucosal papilloma viral DNA replication. Because Crum et al does not teach a method of detecting a precancerous lesion comprising detecting vegetative mucosal papilloma viral DNA replication as indicated by E4 protein expression as required by the instant claims, Crum et al. does not anticipate the invention recited by the newly amended instant claims.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection.

Double Patenting

The office action states that Claims 1-5, 7-12, 14-64 are rejected under the judicially created doctrine of obviousness type double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 6,346,377.

While not necessarily acquiescing to the rejection, Applicant will file a terminal disclaimer upon the indication of allowable claims.

Conclusion

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with

Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

Date: Feb. 10, 2005

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